**LETTER TO JMG**

Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: genetic background of male centenarians is protective against coronary heart disease

D Lio, G Candore, A Crivello, L Scola, G Colonna-Romano, L Cavallone, E Hoffmann, M Caruso, F Licastro, C M Caldarera, A Branzi, C Franceschi, C Caruso

**Key points**

- Centenarians escape, or at least delay, age associated diseases that normally cause mortality at earlier ages. Considerable evidence supports involvement of genetic components to longevity. Accordingly, siblings of centenarians have a marked increased probability of living to 100 years of age. The major trait of the offspring of centenarians is a significantly reduced prevalence of cardiovascular diseases.

- Patients with atherosclerosis have a proinflammatory genotype, and tight control of inflammatory reactions might decrease the incidence of atherosclerosis. Gene polymorphisms for proinflammatory cytokines seem to contribute considerably to the risk of coronary heart disease, including acute myocardial infarction, so alleles associated with susceptibility to acute myocardial infarction expected to be less represented in genetic backgrounds that favour longevity. On the other hand, in Italian centenarian men, the frequency of the genotype associated with interleukin 10 (−1082GG) is associated with significantly increased production of the antiinflammatory cytokine interleukin 10.

- In two different populations from north and south Italy, analysis of genotype distributions showed a significantly higher frequency of the −1082GG genotype among oldest old participants than in controls and patients with acute myocardial infarction. Conversely, the frequency of the −1082AA genotype, associated with low production of interleukin 10, was significantly higher in patients with acute myocardial infarction than in controls and oldest old participants. High production of interleukin 10 thus is protective for acute myocardial infarction and a determinative parameter for longevity.

- People with exceptional longevity possess genetic factors that modulate ageing processes and, in particular, factors protective for cardiovascular disease. This supports the opinion that a genetic background protective against cardiovascular diseases is a component of longevity.

- Our immune system has evolved to control pathogens, so proinflammatory responses are likely to be programmed by evolution to resist fatal infections, and low production of interleukin 10 is associated with an increased resistance to pathogens. Increased concentrations of interleukin 10, however, might better control inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenic complications. These conditions might result in an increased chance of long life in an environment with a reduced load of antigens (that is, pathogens).

Centenarians escape, or at least have a delay in, age associated diseases that normally cause mortality at earlier ages. Considerable evidence supports the involvement of genetic components to longevity. Accordingly, the siblings of centenarians have a markedly increased probability of living to 100 years of age. A recent study showed that the offspring of centenarians had a markedly reduced prevalence of age associated diseases, particularly cardiovascular disease and cardiovascular risk factors. On the other hand, this is not unexpected in view of the fact that cardiovascular diseases account for about 50% of all deaths worldwide.1–3

For a long time, hypercholesterolaemia has been claimed to be the most important risk factor for atherogenesis. Nonetheless, inflammatory processes, coupled with dyslipidaemia and formation of atheroma, have been shown to play an important role in the progression of atherosclerosis. In fact, patients with atherosclerosis have a proinflammatory phenotype, so control of inflammation might have a role in protecting against the complications of atherosclerosis, including acute myocardial infarction.4–6 Acute myocardial infarction may occur as a result of erosion or uneven thinning and rupture of fibrous caps, often at the shoulder of lesions, where macrophages enter, accumulate, and are activated and where apoptosis may occur.7–9

Genetic traits contribute significantly to the risk of coronary heart disease.10 If a number of studies have looked at the hypothesis that variations in genes of the immune system may increase the risk of disease,11–12 Differences in the genetic regulation of inflammatory processes might partially explain why some people develop the disease and why some develop a greater inflammatory response than others. Accordingly, common gene polymorphisms that control high production of inflammatory molecules have been associated with atherosclerosis, and good control of inflammation might play a protective role against atherosclerosis.13–15

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In addition, a variety of studies has shown that interleukin 10 (IL-10) plays a crucial role in the regulation of inflammation. The main function of IL-10 seems to be to limit and ultimately terminate the inflammatory signal in inflammatory cells such as monocytes and macrophages. Stimulation of blood samples from humans with bacterial lipopolysaccharide showed large interindividual variations of production of IL-10, which suggests a genetic component of approximately 75%. Interindividual differences in the regulation of production of IL-10 may be critical with respect to the final outcome of an inflammatory response: that is, within physiological or pathological limits. Furthermore, production of IL-10, independent of the interaction of other cytokine gene products, is controlled genetically by polymorphisms in its own promoter sequence.

We previously reported that the frequency of the homozygous genotype of −1082G for IL-10, included in the −1082G/−819C/−592C haplotype, which is associated with increased production of interleukin and better control of inflammation, is significantly increased in Italian centenarians. To evaluate whether the IL-10 genotype might be a component of a genetic background protective against cardiovascular diseases, we genotyped for interleukin 10 −1082/−819/−592 single nucleotide polymorphisms in two cohorts of men affected by acute myocardial infarction from north and south Italy, two groups of age matched controls, and two groups of oldest old men from the same geographical areas.

METHODS

In this study, we analysed two cohorts of men affected by acute myocardial infarction and unrelated controls matched for age. The cohort comprised 142 patients (mean age 67 years, range 55–80 years) affected by acute myocardial infarction, who were diagnosed at the cardiac unit of Bologna University Hospital, and 153 controls (mean age 67 years, range 65–73 years) from Emilia-Romagna, who had no clinical history of age related disease. The second cohort comprised 90 younger patients (mean age 41 years, range 23–46 years) affected by acute myocardial infarction, who were diagnosed at the cardiac unit of Palermo University Hospital, and 110 healthy controls (mean age 38 years, range 20–55 years) from Sicily. The diagnosis of acute myocardial infarction was based on typical electrocardiographic changes and standard laboratory findings confirmed by echocardiography and coronary angiography.

As a further control, we genotyped samples obtained from two groups of oldest old men (>95 years old and centenarians) without clinical history of cardiovascular diseases: 57 from the same geographical area of Bologna and 52 from Sicily. Age had been verified by researching archival records in the city hall or church registries, or both, with attention paid to the concordance between reported age and personal chronologies (such as age of marriage, age at military service, and age of children). The project was approved by the ethics committees of the two university hospitals, and informed consent was obtained from each participant.

We collected blood specimens in sterile tubes containing tripotassium ethylenediaminetetraacetic acid, and we extracted and processed DNA for IL-10 gene analysis. We identified three different biallelic polymorphisms at −1082 (G→A), −819 (C→T), and −592 (C→A) nucleotides with the −1082, −819, and −592 haplotype specific typing method described by Koss et al. Briefly, we mixed 12 couples of 3’ and 5’ allele specific sequence primer pairs separately in a 13 μl total volume that contained DNA template, 2.00 mM magnesium chloride, 9.8 mM ammonium sulphate, 39.6 mM Tris, 200 μM dNTPs, and 0.2U Taq-polymerase. Cycling was performed at 96°C for 1 minute, followed by five cycles at 96°C for 25 seconds, 70°C for 45 seconds, and 72°C for 45 seconds, 20 cycles at 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds, and five cycles at 96°C for 25 seconds, 55°C for 60 seconds, and 72°C for 120 seconds. Products of polymerase chain reaction that potentially contained the −592/−819, −592/−1082, or −819/−1082 possible allele combinations were detected by electrophoresis on 2% agarose.

Interleukin 10 haplotype and genotype frequencies were evaluated by gene count. The data were tested for goodness of fit between the observed and expected genotype values (χ² test) and their fit to Hardy-Weinberg equilibrium. Chi squared tests (3×2 tables, or 2×2 table with Yates’ correction) were performed to calculate significant different haplotype or genotype distribution between patients with acute myocardial infarction, age matched controls and oldest old controls. As multiple comparisons were made, Bonferroni’s correction was applied and p values were multiplied by the number of haplotypes detected (that is, 3). The strength of the statistical association was expressed by odds ratio of risk and 95% of confidence intervals.

RESULTS

Direct typing of IL-10 haplotypes confirmed the presence of the only three possible allele combinations characteristic of IL-10 polymorphisms in Caucasian people, which allowed us to present the results as −1082/−819/−592 haplotype frequencies.

Table 2 shows the genotype frequencies for −1082/−819/−592 IL-10 haplotypes in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Significantly different distributions of −1082/−819/−592 haplotypes were observed among controls and old (mean age 67 years) men with acute myocardial infarction (p = 0.0027), among oldest old and old patients with acute myocardial infarction (p = 0.0003) and among controls and oldest old (p = 0.039) from north Italy. Marginally significant different distributions of −1082/−819/−592 haplotypes were observed among controls and young (mean age 41 years) men with acute myocardial infarction (p = 0.069) and controls and oldest old (p = 0.078), whereas a highly significant difference was observed among oldest old and patients with acute myocardial infarction (p = 0.0006) from South Italy.

Table 3 shows IL-10 −1082G→A genotype frequencies in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Significant different distributions of −1082G→A genotypes between Bologna and Sicily are shown in Table 3.
were observed among controls and men with acute myocardial infarction (p = 0.0003), among controls and oldest old (p = 0.0024) and among oldest old men with acute myocardial infarction (p = 0.0003) from north Italy. In the same population, the analysis of the single genotype distribution showed a higher frequency of the −1082GG homozygous genotype in oldest old men than controls (p = 0.0002; odds ratio 3.20 (95% CI 1.72 to 6.07)) and men with acute myocardial infarction (p = 0.0002; 3.10 (2.90 to 20.20)). The frequency of the −1082AA homozygous genotype was significantly higher in men with acute myocardial infarction than in controls (p = 0.0001; 3.00 (1.78 to 5.04)) and oldest old men (p = 0.019) and among oldest old men with acute myocardial infarction (p = 0.0003) from south Italy. In the same population, the analysis of the single genotype distribution showed a significant increase of −1082GG homozygous genotype frequency in oldest old men with respect to the controls (p = 0.0034; 3.20 (1.72 to 6.07)) and men with acute myocardial infarction (p = 0.0005; 3.97 (1.86 to 8.48)). Finally, the frequency of −1082AA was significantly higher in men with acute myocardial infarction than in controls (p = 0.0007; 2.80 (1.54 to 5.08)) and oldest old (p = 0.0001; 8.99 (3.27–24.70)).

**DISCUSSION**

The highly polymorphic IL-10 gene is located on chromosome 1 at 1q31–32. Several polymorphisms located close to or within the IL-10 gene can influence the transcription levels of mRNA coding for the protein. Three polymorphisms in the proximal region of IL-10 gene have been described, but conflicting results have been reported on the influence of self-standing or reciprocal linkage on IL-10 transcription.

Three well documented haplotypes, involving three single nucleotide polymorphisms at −1082 (G→A), −819 (C→T), and −592 (C→A) nucleotides of promoter, have been identified in Caucasians, and our results confirm the above mentioned results in Italian population samples. In the original paper of Turner et al., the −1082 SNP was

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**Table 2** Genotype frequencies for −1082/−819/−592 IL-10 haplotypes in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Values are numbers (percentages)

<table>
<thead>
<tr>
<th>Participants</th>
<th>No. of participants</th>
<th>Genotype</th>
<th>GCC/GCC</th>
<th>GCC/ACC</th>
<th>GCC/ATA</th>
<th>ACC/ACC</th>
<th>ACC/ATA</th>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>“Old” patients with acute myocardial infarction</td>
<td>142†</td>
<td>30 (21.1)</td>
<td>35 (24.6)</td>
<td>17 (12.0)</td>
<td>20 (14.1)</td>
<td>26 (18.3)</td>
<td>14 (9.9)</td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>153‡</td>
<td>48 (31.4)</td>
<td>41 (26.8)</td>
<td>34 (22.2)</td>
<td>12 (7.8)</td>
<td>10 (6.5)</td>
<td>8 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Oldest old men</td>
<td>57</td>
<td>34 (59.6)</td>
<td>10 (17.5)</td>
<td>8 (14.0)</td>
<td>2 (3.5)</td>
<td>2 (3.5)</td>
<td>1 (1.8)</td>
<td></td>
</tr>
<tr>
<td>South Italy</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Young” patients with acute myocardial infarction</td>
<td>90***</td>
<td>17 (18.9)</td>
<td>17 (18.9)</td>
<td>12 (13.3)</td>
<td>16 (17.8)</td>
<td>19 (21.1)</td>
<td>9 (10.0)</td>
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<tr>
<td>Male controls</td>
<td>110‡</td>
<td>26 (23.6)</td>
<td>30 (27.3)</td>
<td>26 (23.6)</td>
<td>10 (9.1)</td>
<td>10 (9.1)</td>
<td>8 (7.3)</td>
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</tr>
<tr>
<td>Oldest old men</td>
<td>52</td>
<td>25 (48.1)</td>
<td>12 (23.1)</td>
<td>11 (21.2)</td>
<td>2 (3.8)</td>
<td>3 (5.8)</td>
<td>1 (1.9)</td>
<td></td>
</tr>
</tbody>
</table>

Haplotypes were determined with sense and anti-sense primers specific for the three single nucleotide polymorphisms considered, which allowed direct typing of the existing haplotypes. For comparison of genotype distribution, χ2 test with Yates’ correction was performed (Graphpad Instat, Graphpad Software, San Diego, CA, USA). Bonferroni’s correction was applied by multiplying the obtained p values by three possible haplotypes.

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**Table 3** Interleukin 10 −1082G→A genotype frequencies in men with acute myocardial infarction, age matched controls, and oldest old controls from north and south Italy. Values are numbers (percentages)

<table>
<thead>
<tr>
<th>Participants</th>
<th>No. of participants</th>
<th>Genotype</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Italy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Old” patients with acute myocardial infarction</td>
<td>142†</td>
<td>30 (21.1)</td>
<td>52 (36.6)</td>
<td>60 (42.2)</td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>153‡</td>
<td>48 (31.4)</td>
<td>75 (49.0)</td>
<td>30 (19.6)</td>
<td></td>
</tr>
<tr>
<td>Oldest old men</td>
<td>57</td>
<td>34 (59.6)</td>
<td>18 (31.6)</td>
<td>5 (8.8)</td>
<td></td>
</tr>
<tr>
<td>South Italy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Young” patients with acute myocardial infarction</td>
<td>90***</td>
<td>17 (18.9)</td>
<td>29 (32.2)</td>
<td>44 (48.9)</td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>110‡</td>
<td>26 (23.6)</td>
<td>56 (50.9)</td>
<td>28 (25.5)</td>
<td></td>
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<tr>
<td>Oldest old men</td>
<td>52</td>
<td>25 (48.1)</td>
<td>23 (44.2)</td>
<td>6 (11.5)</td>
<td></td>
</tr>
</tbody>
</table>

The genotype percentages were obtained considering the following haplotype combinations: (A) −1082G/−819C/−592C homozygous participants for −1082GG genotype; (B) −1082A/−819C/−592C −1082G/−819C/−592C and −1082A/−819T/−592A −1082G/−819C/−592C heterozygous participants for −1082GA genotypes; (C) −1082A/−819C/−592C and −1082A/−819T/−592A −1082G/−819C/−592C homozygous and −1082A/−819C/−592C −1082A/−819T/−592A −1082A/−819T/−592A heterozygous participants for −1082AA genotypes. For the comparison of genotype distribution, χ2 test with Yates’ correction was performed (Graphpad Instat, Graphpad Software, San Diego, CA, USA). Bonferroni’s correction was applied by multiplying the obtained p values by three possible haplotypes.

†p = 0.0003 (vs. male controls); ‡p = 0.0003 (vs. oldest old men); ††p = 0.0024 (vs. oldest old men); †††p = 0.0069 (vs. male controls); ‡‡p = 0.0006 (vs. oldest old men); ‡‡‡p = 0.078 (vs. oldest old men).
Interleukin 10, inflammation, infarction, and longevity

Interleukin 10 (IL-10) is a cytokine that has been shown to have anti-inflammatory effects and is involved in the regulation of immune responses. It is detectable in human atherosclerotic lesions and is involved in the progression of atherosclerosis. Knockout mice deficient for IL-10 have a 30-fold higher susceptibility to atherosclerosis, induced either by high cholesterol diet or by cardiac allograft vasculopathy. These findings suggest that IL-10 has an inhibitory effect on atherosclerosis.

Previous studies have shown that people with the IL-10 high-producer genotype effectively may be protected against developing atherosclerosis. In a study of patients with coronary heart disease, the IL-10 high-producer genotype was associated with a lower prevalence of hypertension, coronary heart disease, metabolic syndrome, and increased homocysteine for the 1405V variant in the cholesteryl ester transfer protein gene, which is involved in regulation of lipoprotein and its particle sizes.

It is intriguing that in another population with different style of life—Ashkenazi Jews—people with exceptional longevity and their offspring had a genetic background that conditioned significantly larger particle sizes of high density lipoprotein and LDL. Again, this phenotype was associated with a lower prevalence of hypertension, coronary heart disease, metabolic syndrome, and increased homocysteine for the 1405V variant in the cholesteryl ester transfer protein gene, which is involved in regulation of lipoprotein and its particle sizes.

In addition, centenarians, who have overcome the age-related risk, may be a better control group for case-control studies focused on age-associated diseases with multifactorial etiology. It is, in fact, noteworthy that the −1082GG genotype, which is associated with an increased production of IL-10 and has been associated with the possibility of reaching the extreme limits of human lifespan in Italian men, was underrepresented in young and old patients with coronary heart disease, with the −1082AA genotype being overrepresented.

Finally, our data prompt consideration of the role that antagonistic pleiotropy plays in diseases and longevity. In fact, our immune system has evolved to control pathogens, so proinflammatory responses are likely to be evolutionarily programmed to resist fatal infections, and low production of IL-10 is associated with an increased resistance to pathogens. However, increased levels of IL-10 might better control inflammatory responses induced by chronic vessel damage and reduce the risk of atherogenic complications. These conditions might result in an increased chance of long life survival in an environment with reduced antigen (that is, pathogen) load.

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Biological and genetic markers of successful and unsuccessful diseases prevention in ageing: the model of centenarians.


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